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**13. ABSTRACT** ***(Maximum 200 Words)***

Several reproductive factors have been consistently associated with increased risk of breast cancer in the general population. These associations are less well characterized in women with a family history of the disease. The purpose of this research was to examine the association of reproductive factors with breast cancer risk among sisters, daughters, granddaughters, and nieces of 426 breast cancer probands as well as among women who married into the 426 families. The risk of breast cancer associated with age at menarche, age at menopause, parity, age at first birth, age at last birth, and infertility was not significantly modified by family history. However, our results suggest that use of early formulations of oral contraceptives by women with a strong family history may further elevate their breast cancer risk. The association was particularly strong in families with multiple cases of breast and ovarian cancer. We were not able to draw conclusions regarding use of more recent low dose oral contraceptives. Women in high-risk families have been counseled to take oral contraceptives to reduce their risk of ovarian cancer. Further research is needed to ensure this does not come at the cost of an increased risk of breast cancer.

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## Introduction

Several reproductive factors, including an early age at menarche, nulliparity, late age at first birth, and late age at menopause have been consistently associated with an increased risk of breast cancer. The association of these factors in addition to other reproductive and fertility factors with risk of breast cancer is less well characterized in women with a family history of breast cancer. The scope of this research was to examine the association of reproductive and fertility factors with risk of breast cancer among sisters, daughters, granddaughters, and nieces of 426 breast cancer probands as well as among women who married into the 426 families. Variables examined included oral contraceptive use, age at menarche, age at menopause, parity, age at first and last birth, difficulty becoming pregnant, and reason for difficulty becoming pregnant. The results of this research could have important implications for breast cancer prevention and early detection in women with a family history of breast cancer.

## Body

Funding for this grant ended September 30, 2000. To date, one paper has been published, one abstract has been presented, and one abstract has been submitted. A second manuscript is being drafted.

Results of the oral contraceptive analyses were published in the October 11, 2000 issue of the Journal of the American Medical Association. This study was the first multigenerational family study to examine the association between oral contraceptive use and breast cancer. While oral contraceptives have been shown to be weakly associated with breast cancer in the general population, studies have been inconsistent in regards to the effect of oral contraceptives on breast cancer risk in women with a family history of the disease. The results of our research suggest that it is the subset of women who have a strong family history of breast cancer who may be at further increased risk. Ever use of oral contraceptives was associated with a 3.3-fold (95% C.I.: 1.6-6.7) increased risk of breast cancer among sisters and daughters of breast cancer probands. Oral contraceptives were not associated with significantly increased risk of breast cancer among granddaughters and nieces of probands (RR=1.2; 95% C.I.: 0.8-2.0) or among marry-ins (RR=1.2; 95% C.I.: 0.8-1.9). The test for interaction between oral contraceptive use and relationship to proband was statistically significant (p=0.03). Results were essentially unchanged after adjustment for parity, age at first birth, age at menarche, age at menopause, oophorectomy, smoking, and education.

To study families most likely to be carrying a mutation in BRCA1 or BRCA2, analyses were conducted in high-risk families defined by the number of breast and ovarian cancers among the blood relatives. Among 132 high risk families in which at least 3 blood relatives were diagnosed with breast or ovarian cancer, the interaction of oral contraceptive use with degree of relationship reached even stronger statistical significance (p=0.006) than in the entire cohort of 426 families. Among sisters and daughters, ever use was associated with a relative risk of 4.6 (95% C.I.: 2.0-10.7). Use of oral contraceptives by granddaughters and nieces was not associated with significantly increased risk of breast cancer (RR=1.2; 95% C.I.: 0.7-2.0). When the analysis was conducted in 35 very high risk families in which at least 5 blood relatives were diagnosed with breast or ovarian cancer, the risk among sisters and daughters was even greater

(relative risk=11.4; 95% C.I.: 2.3-56.4). No association was seen among granddaughters and nieces (RR=1.4; 95% C.I.: 0.6-3.3).

We questioned whether the elevated risk of breast cancer associated with oral contraceptive use in sisters and daughters of the probands was due to these individuals being more likely to have been exposed to the earlier formulations of oral contraceptives that contained higher doses of estrogen and progestins. The amount of estrogen in oral contraceptives has decreased from approximately 150 micrograms to less than 50 micrograms currently, with concurrent decreases in the level of progestins. Although we did not ascertain specific formulations of oral contraceptives used, we did collect data on the particular years of oral contraceptive use. With these data, we examined estimated years of exposure to high dose and years of exposure to low dose formulations. Since all oral contraceptives initially marketed after 1975 contain less than 50 micrograms of ethinyl estradiol and 1 mg or less of several progestins, we used this year as the cutpoint. No association was observed between oral contraceptive use after 1975 and risk of breast cancer for any category of family history, although statistical power was limited. However, the risk of breast cancer associated with oral contraceptive use prior to 1975 was elevated among women with a first degree family history of breast cancer (relative risk=3.3; 95% C.I.: 1.5-7.2), but not among women with a second degree family history (relative risk=1.3; 95% C.I.: 0.8-2.0) or among marry-ins (relative risk=1.2; 95% C.I.: 0.8-1.9).

Our results suggest that the use of early formulations of oral contraceptives by women with a strong family history of breast cancer may further elevate their breast cancer risk. Because the mean age at interview of women who used oral contraceptives after 1975 was only 43 years, further follow-up is needed to investigate any association between current formulations of oral contraceptives and breast cancer in high-risk women.

Observational studies have demonstrated a reduction in risk of ovarian cancer with oral contraceptive use. As a result, women from high-risk breast-ovarian cancer families are often counseled to take oral contraceptives to reduce their ovarian cancer risk. Our results provide additional information for these women and their physicians to consider. Although it was the early formulations of oral contraceptives that were associated with increased breast cancer risk, it will be important to ensure that the reduction in ovarian cancer risk with current formulations does not come at the expense of an increased risk of breast cancer as well.

The association between other reproductive factors and breast cancer by relationship to proband is presented in Table 1. Results are presented for self-respondents only. Parity, age at first birth, and age at last birth were also collected from surrogates of deceased subjects or those not able to complete a telephone interview. Results were not substantially different when all women were included. [Note: DES was not examined because there were only a total of 7 breast cancer cases among 88 women reporting exposure. Use of Clomid was also reported by a small number of women (n=72) and none of these reported breast cancer.] No statistically significant interactions were observed between these reproductive factors and relationship to proband. In general, the effects were similar for sisters, daughters, nieces, granddaughters, and marry-ins.

In conclusion, the risk of breast cancer associated with endogenous correlates of hormonal exposure, namely age at menarche, age at menopause, parity, age at first birth, age at last birth, and infertility does not appear to be significantly modified by family history. However, women with a strong family history appear to be at further increased risk of breast cancer if they have been exposed to early formulations of oral contraceptives. Close surveillance of these women will be important to increase the probability of early detection of any breast cancers that do develop.

Additional questions have been raised as a result of these findings. Namely, what is the effect of oral contraceptive use in women carrying mutations in BRCA1 or BRCA2? Is the risk of breast cancer increased by use of recent, low dose oral contraceptives in women with a strong family history? These questions are currently being addressed in this same cohort of families through a grant from the National Cancer Institute.

Table 1. Association of reproductive and fertility factors with breast cancer by relationship to proband*

| **Risk Factor** | **Sisters/Daughters n=395** | **Niece/Granddaughters n=3014** | **Marry-ins n=2768** |
|---|---|---|---|
| | **RR (95% C.I.)** | **RR (95% C.I.)** | **RR (95% C.I.)** |
| **Age at menarche** | | | |
| <12 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 12-14 | 0.4 (0.2-0.8) | 1.0 (0.6-1.7) | 0.8 (0.5-1.4) |
| 15+ | 0.3 (0.1-0.8) | 0.8 (0.4-1.5) | 0.6 (0.3-1.4) |
| **Age at menopause[a]** | | | |
| ≤50 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| >50 | 1.5 (0.7-2.9) | 2.4 (1.4-3.9) | 1.2 (0.7-2.1) |
| **Reason for menopause[b]** | | | |
| Natural | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| Bilateral oophorectomy | 0.7 (0.2-3.0) | 1.1 (0.6-2.0) | 1.6 (0.8-3.1) |
| Other | 2.5 (1.2-5.4) | 1.3 (0.8-2.2) | 1.9 (1.1-3.3) |
| **Parity/Age at first birth** | | | |
| Nulliparous | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 1-2, ≤20 | 1.7 (0.4-7.2) | 1.6 (0.8-3.4) | 0.4 (0.1-1.5) |
| 1-2, >20 | 1.4 (0.5-3.7) | 0.6 (0.3-1.1) | 0.5 (0.2-1.2) |
| 3+, ≤20 | 1.0 (0.3-3.3) | 0.7 (0.4-1.4) | 0.6 (0.3-1.4) |
| 3+, >20 | 0.8 (0.3-2.3) | 0.7 (0.4-1.2) | 0.7 (0.3-1.7) |
| **Parity/Age at last birth[c]** | | | |
| Nulliparous | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 1-2, <30 | 1.5 (0.5-4.5) | 0.9 (0.5-1.8) | 0.5 (0.2-1.3) |
| 1-2, ≥30 | 1.3 (0.5-3.8) | 0.5 (0.2-1.1) | 0.5 (0.2-1.4) |
| 3+, <30 | 0.9 (0.3-3.2) | 0.9 (0.4-1.7) | 0.5 (0.2-1.3) |
| 3+, ≥30 | 0.9 (0.3-2.5) | 0.6 (0.4-1.1) | 0.7 (0.3-1.6) |
| **Infertility[c]** | | | |
| No | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| Yes | 1.1 (0.4-2.8) | 0.7 (0.4-1.3) | 1.2 (0.7-2.0) |
| **Reason for infertility[c,d]** | | | |
| No infertility | All Blood Relatives: | 1.0 (ref) | 1.0 (ref) |
| Hormonal | | 0.8 (0.2-3.5) | 0.7 (0.2-2.8) |
| Non-hormonal | | 0.6 (0.1-2.2) | 1.0 (0.4-2.6) |
| Partner | | 1.9 (0.5-8.2) | 0.5 (0.1-3.5) |
| DK, no clinic visit | | 1.5 (0.8-2.8) | 0.8 (0.5-1.5) |

*Analyses adjusted for parity/age at first birth, age at menarche, age at menopause, oral contraceptive use, education, smoking, and alcohol

[a] Also adjusted for reason for menopause and HRT

[b] Also adjusted for HRT

[c] Not adjusted for age at first birth because some women only had one child

[d] Analyses conducted among women who reported being married or in a marriage-like relationship

[e] All blood relatives combined due to small numbers

## Key Research Accomplishments

- Women with a strong family history of breast cancer who used early formulations of oral contraceptives appear to be at further increased risk of developing breast cancer.
- The risk of breast cancer associated with age at menarche, age at menopause, parity, age at first birth, age at last birth, and infertility was not significantly modified by family history.
- The oral contraceptive findings were presented as a poster at the 49th Annual Meeting of the American Society of Human Genetics, and were published in the October 11, 2000 issue of the Journal of the American Medical Association.
- An abstract on the association of age at menarche, age at menopause, parity, and age at first birth by relationship to proband has been submitted for the 92nd Annual Meeting of the American Association for Cancer Research.

## Reportable Outcomes

*Manuscripts*

Grabrick DM, Hartmann LC, Cerhan JR, Vierkant RA, Therneau TM, Vachon CM, Olson JE, Couch FJ, Anderson KE, Pankratz S, Sellers TA. Risk of breast cancer with oral contraceptive use in women with a family history of breast cancer. JAMA 284(14):1791-1798.

*Abstracts*

Grabrick DM, Cerhan JR, Couch FJ, Vierkant RA, Therneau TM, Vachon CM, Olson JE, Pankratz VS, Hartmann LC, Sellers TA. Association of oral contraceptives with breast cancer risk in a population-based sample of 426 breast cancer families. 49th Annual Meeting of the American Society of Human Genetics, October 1999.

Grabrick DM, Cerhan JR, Vierkant RA, Sellers TA. Association of endogenous correlates of hormonal exposure with breast cancer risk in 426 breast cancer families. Submitted: 92nd Annual Meeting of the American Association for Cancer Research.

# Risk of Breast Cancer With Oral Contraceptive Use in Women With a Family History of Breast Cancer

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**Context** Oral contraceptive (OC) use is weakly associated with breast cancer risk in the general population, but the association among women with a familial predisposition to breast cancer is less clear.

**Objective** To determine whether the association between OC use and risk of breast cancer is influenced by family history of the disease.

**Design and Setting** Historical cohort study of 426 families of breast cancer probands diagnosed between 1944 and 1952 at the Tumor Clinic of the University of Minnesota Hospital. Follow-up data on families were collected by telephone interview between 1991 and 1996.

**Participants** A total of 394 sisters and daughters of the probands, 3002 granddaughters and nieces, and 2754 women who married into the families.

**Main Outcome Measure** Relative risk (RR) of breast cancer associated with history of OC use by relationship to proband.

**Results** After accounting for age and birth cohort, ever having used OCs was associated with significantly increased risk of breast cancer among sisters and daughters of the probands (RR, 3.3; 95% confidence interval [CI], 1.6-6.7), but not among granddaughters and nieces of the probands (RR, 1.2; 95% CI, 0.8-2.0) or among marry-ins (RR, 1.2; 95% CI, 0.8-1.9). Results were essentially unchanged after adjustment for parity, age at first birth, age at menarche, age at menopause, oophorectomy, smoking, and education. The elevated risk among women with a first-degree family history of breast cancer was most evident for OC use during or prior to 1975, when formulations were likely to contain higher dosages of estrogen and progestins (RR, 3.3; 95% CI, 1.5-7.2). A small number of breast cancer cases (n=2) limited the statistical power to detect risk among women with a first-degree relative with breast cancer and OC use after 1975.

**Conclusions** These results suggest that women who have ever used earlier formulations of OCs and who also have a first-degree relative with breast cancer may be at particularly high risk for breast cancer. Further studies of women with a strong family history who have used more recent lower-dosage formulations of OCs are needed to determine how women with a familial predisposition to breast cancer should be advised regarding OC use today.

*JAMA. 2000;284:1791-1798* www.jama.com

IN GENERAL POPULATION SAMPLES, oral contraceptives (OCs) have been observed to be weakly associated with risk of breast cancer up to 10 years after a woman discontinues use.[1] Much less is known, however, regarding this association among women with a familial predisposition to breast cancer; while some studies have shown a higher risk among women with a family history,[2-6] others have found little or no such evidence.[7-18] Observational studies have demonstrated a reduction in risk of ovarian cancer with OC use. As a result, women from high-risk breast-ovarian cancer families are often counseled to take OCs to reduce their ovarian cancer risk.[19,20] However, a small study of Ashkenazi Jewish women with breast cancer suggests that OC use may increase the risk

**For editorial comment see p 1837.**

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of breast cancer more in carriers of *BRCA1* or *BRCA2* mutations than in noncarriers.[21]

Since a family history of breast cancer may not only reflect shared genes but also shared exposures, a family study that incorporates carefully ascertained risk factor data is a robust approach for examining the potential interaction of OC use with family history. We evaluated the association between OC use and breast cancer risk according to family history of the disease in a large historical cohort of Minnesota families. We include in our analysis data on the total duration and dates of OC use, ages of exposure to OCs, and potential confounding factors. To our knowledge, this is the first study to examine this interaction in the context of a multigenerational family study.

## METHODS

### Study Population

Details of the study design and methods have been published elsewhere.[22] Briefly, this study originated from a case-control family study initiated in 1944 at the Dight Institute for Human Genetics at the University of Minnesota, Minneapolis.[23] A consecutive series of 544 women diagnosed with breast cancer was ascertained between 1944 and 1952 to examine the influence of childbearing, breastfeeding, and hereditary susceptibility on the risk of breast cancer. At that time, probands were asked to provide the names, addresses, and cancer history of their children, siblings, nieces, and nephews.

After this initial study, the records on these families remained in storage, untouched for nearly 50 years, until a follow-up study was conducted between 1991 and 1996.[22] Of 544 families in the cohort at the start of follow-up in 1952, we excluded 40 because the proband had prevalent breast cancer (diagnosed before 1940) and 42 because no or very few relatives were alive at start of follow-up. Of the remaining 462 families, 20 were lost to follow-up, 10 had no living members in the sampling frame, and 6 refused to participate. A total of 426 families (92.2% after baseline exclusions) were successfully updated. Adult sisters, daughters, granddaughters, nieces, and marry-ins were eligible for the current study.

### Data Collection

Data on cancer history and risk factors for breast cancer were collected through telephone interviews. The participation rate of self-respondents in the telephone interview was 93% (6194/6664). Selected data including cancer history were obtained through surrogate respondents for 2656 (96%) of 2778 women who were deceased. In addition, selected data were obtained from surrogates of 361 (96%) of 376 women who were living but incapable of responding to a telephone interview. Only 568 women in the 426 families were completely lost to follow-up.

We examined the accuracy of self-reporting of breast cancer by reviewing medical records, pathology reports, and death certificates for a sample of 138 self-reports and were able to confirm 99% of these cases of breast cancer. To increase validity of reports, we collected data on OC use only from self-respondents. We questioned them regarding ever vs never use of OCs, age use began, and age use stopped. The main analyses were thus conducted among adult sisters, daughters, granddaughters, nieces, and marry-ins in these families who participated in the telephone interview; data were also collected from surrogate respondents to help evaluate potential bias.

All subjects provided verbal informed consent, and the protocol was reviewed and approved by the University of Minnesota Institutional Review Board.

### Statistical Analyses

Analyses were performed using Cox proportional hazards regression.[24] Exclusions were made for cancers (other than skin) diagnosed before baseline (defined as proband's date of breast cancer diagnosis). Follow-up began at age 18 years or age when the proband in the family was diagnosed, whichever was later. Follow-up continued until age at breast cancer diagnosis or age at interview, whichever came first.

Survival was modeled as a function of age, since age is a better predictor of breast cancer risk than is length of follow-up time in this study.[25] Oral contraceptive use was modeled as a time-dependent variable. Only OC exposure occurring prior to breast cancer diagnosis was included. Analyses were stratified by birth cohort to control for potential cohort effects in OC use and breast cancer incidence. In addition, we accounted for the nonindependence of observations within families by using a robust variance estimate.[26]

The overall association of OC use with breast cancer risk in the entire cohort was examined first. Subsequent analyses evaluated whether the degree of relationship to the proband modified the effect of OC use on breast cancer risk. Never OC users were defined as the reference group for each category of relationship to the proband.

Since granddaughters and nieces may have a closer affected relative than the original proband in the family, analyses of OC use were also run with degree of relationship redefined as the closest affected relative. This resulted in 176 granddaughters and nieces being reclassified into the highest risk category. The results were essentially unchanged. Therefore, analyses define family history as relationship to the proband unless otherwise specified.

Potential confounding variables were evaluated for each model after allowing for the interaction of relationship to proband with OC use. A variable was considered a confounder if its addition changed the hazard ratio for any of the OC-use-by-relationship variables by more than 10%. There was no evidence for confounding by the following variables: parity and age at first birth, education, age at menarche, age at menopause, oophorectomy, lifetime alcohol intake, and body mass index. Diabetes, smoking, and fibroid tumors of the uterus, possible contraindications for OC use, were also ruled out as confounders. Polycystic ovaries and endometriosis, possible indications for OC use, were

**Table 1.** Description of a Cohort of 426 Families Ascertained Through Probands Diagnosed With Breast Cancer at the University of Minnesota Between 1944 and 1952*

| Variable | Relationship to Proband | | | | | |
|---|---|---|---|---|---|---|
| | Sisters (n = 72) | Daughters (n = 322) | Granddaughters (n = 1427) | Nieces (n = 1575) | Marry-ins (n = 2754) | Total (n = 6150) |
| Birth cohort, No. (%) | | | | | | |
| Before 1913 | 30 (41.7) | 30 (9.3) | 3 (0.2) | 133 (8.4) | 143 (5.2) | 339 (5.5) |
| 1913-1925 | 38 (52.8) | 130 (40.4) | 65 (4.6) | 590 (37.5) | 639 (23.2) | 1462 (23.8) |
| 1926-1941 | 4 (5.6) | 140 (43.5) | 339 (23.8) | 592 (37.6) | 955 (34.7) | 2030 (33.0) |
| After 1941 | 0 (0) | 22 (6.8) | 1020 (71.5) | 260 (16.5) | 1017 (36.9) | 2319 (37.7) |
| Mean age (range), y† | 79.0 (62-93) | 67.6 (36-89) | 45.3 (18-84) | 65.0 (20-95) | 57.5 (21-94) | 57.4 (18-95) |
| No. of breast cancers‡ | 6 | 32 | 24 | 91 | 86 | 239 |
| Mean age at breast cancer onset (range), y | 60.0 (50-73) | 56.6 (34-83) | 50.4 (25-72) | 57.0 (26-81) | 57.5 (27-82) | 56.5 (25-83) |

*Percentages may not add to 100 due to rounding.
†At time of interview.
‡Diagnosed between 1952 and 1996.

evaluated as potential confounders, but they were not found to influence the results either. In addition to evaluating potential confounders on an individual basis, we fit multivariate models with simultaneous adjustment for parity, age at first birth, age at menarche, age at menopause, oophorectomy, pack-years of smoking, and education. Since the risk ratios generally changed by less than 10% in these multivariate models, we have presented the most parsimonious models, unadjusted for these variables but accounting for age, birth cohort, and nonindependence of observations within a family. Any meaningful changes upon adjustment are presented in the results.

Although collection of data on OC use was limited to self-respondents, selected information was collected through surrogate respondents for 96% of female family members who had died as well as for 96% of living women who were unable to complete a telephone interview. This information was used to try to control for potential biases due to missing data on OC use by means of a propensity score method.[27,28] A variable was created to designate missing vs nonmissing OC use data. The following variables were then fit as predictors of nonmissing OC use data in a logistic regression model: education, alcohol use, cigarette smoking, diabetes, cancer, degree of relationship to the proband, age at start of follow-up, and birth cohort. The resulting coefficients were used to estimate the probability of nonmissing OC data for each woman. The original Cox proportional hazards model restricted to women with nonmissing OC data was then refit using the inverse of this probability as a weighting factor.[29] People with a high probability of missing OC use were thus weighted more heavily in the Cox model because they were underrepresented in the cohort. Data analyses were performed using the SAS (SAS Institute Inc, Cary, NC) and Splus (Mathsoft Inc, Seattle, Wash) software systems.

**Table 2.** Characteristics of Oral Contraceptive (OC) Use by Relationship to Proband in a Cohort of 426 Families*

| Variable | Relationship to Proband | | |
|---|---|---|---|
| | Sisters and Daughters (n = 394) | Nieces and Granddaughters (n = 3002) | Marry-ins (n = 2754) |
| Never used | 303 (76.9) | 1350 (45.0) | 1341 (48.7) |
| Current users | 0 (0) | 135 (4.5) | 71 (2.6) |
| Former users | 91 (23.1) | 1517 (50.5) | 1342 (48.7) |
| Age at first OC use, mean (SD) [range], y | 30.1 (7.1) [17-54] | 23.8 (6.8) [11-62] | 24.5 (6.8) [11-52] |
| Age at end of OC use, mean (SD) [range], y† | 35.6 (7.4) [22-55] | 30.5 (8.1) [14-62] | 30.8 (8.0) [15-65] |
| Duration of OC use, mean (SD) [range], y† | 6.0 (5.8) [0.5-29.5] | 7.2 (5.9) [0.5-30] | 6.8 (5.8) [0.5-37.5] |

*Data are presented as number (percentage) unless otherwise indicated.
†Includes current users.

## RESULTS

### Description of the Cohort

The age at diagnosis of breast cancer among the original probands showed wide variation, ranging from 21 to 88 years. This is reflected in the birth cohorts of the relatives (TABLE 1). The study cohort consists of 3396 blood relatives and 2754 marry-ins (6150 total). Breast cancer occurred in 153 of the blood relatives and 86 of the marry-ins during the follow-up period, after 1952. The age at onset of breast cancer ranged from 25 to 83 years. The mean length of follow-up was 36.6 years.

In the study cohort, the lifetime prevalence of ever having used OCs was 51% overall and was similar for blood relatives and marry-ins ($P=.99$); 6.5% of ever users reported current use of OCs. Among women who ever took OCs, the average length of use was 7.0 years (range, 0.5-37.5 years).

TABLE 2 describes OC use by relationship to the proband. Sisters and daughters of the proband were less likely to have used OCs than were nieces, granddaughters, and marry-ins, and were more likely to start and end OC use at later ages. The duration

of use did not markedly differ by relationship but was slightly lower among sisters and daughters.

**Table 3.** Distribution of Breast Cancer Risk Factors by Oral Contraceptive Use in a Cohort of 426 Families, 1991-1996*

| Risk Factors | Oral Contraceptive Use: Ever (n = 3156) | Oral Contraceptive Use: Never (n = 2994) |
|---|---|---|
| Parity, age at first birth, y | | |
| Nulliparous | 358 (11.4) | 377 (12.6) |
| 1-2, ≤20 | 355 (11.3) | 182 (6.1) |
| 1-2, >20 | 942 (29.9) | 770 (25.7) |
| ≥3, ≤20 | 726 (23.0) | 589 (19.7) |
| ≥3, >20 | 773 (24.5) | 1073 (35.9) |
| Age at menarche, mean (SD), y | 12.9 (1.5) | 13.1 (1.6) |
| Menopausal status | | |
| Premenopausal | 1605 (51.5) | 253 (8.6) |
| Age at menopause <44 y | 657 (21.1) | 772 (26.2) |
| Age at menopause 45-50 y | 518 (16.6) | 1045 (35.5) |
| Age at menopause >50 y | 338 (10.8) | 876 (29.7) |
| Oophorectomy | 348 (11.0) | 544 (18.2) |
| Smoking history | | |
| Never smoked | 1439 (45.8) | 1842 (62.0) |
| ≤20 pack-years | 952 (30.3) | 510 (17.2) |
| >20 pack-years | 754 (24.0) | 620 (20.9) |
| Education | | |
| <High school graduate | 369 (11.7) | 888 (29.7) |
| High school graduate | 1170 (37.1) | 1058 (35.4) |
| Some college | 1054 (33.4) | 751 (25.1) |
| College graduate | 562 (17.8) | 294 (9.8) |

*Data are presented as number (percentage) unless otherwise indicated. Percentages may not add to 100 due to rounding. Distribution of each risk factor differs significantly by oral contraceptive use, $P<.001$.

TABLE 3 shows the distribution of breast cancer risk factors by OC use. Women who had ever used OCs were much more likely to be premenopausal at the time of interview than women who had never used OCs (52% vs 9%). Oophorectomy was slightly less common among OC users, while smoking was more common among users than nonusers. Oral contraceptive users also tended to have a higher level of education.

### Association of OCs With Breast Cancer

Among the entire cohort, ever use of OCs was associated with a relative risk (RR) of 1.4 (95% CI, 1.0-2.0) for breast cancer. Risk did not differ by duration of use (defined by the median split). The RR associated with 1 to 4 years of OC use vs never use was 1.5 (95% CI, 1.0-2.3), while greater than 4 years of use conferred a RR of 1.3 (95% CI, 0.9-1.9).

### Modification of the OC–Breast Cancer Association by Relationship to Breast Cancer Probands

To determine if the apparent risk associated with OC use was modified by genetic background, analyses were performed within strata defined by relationship to the proband (TABLE 4). Never users served as the reference group within each stratum. In the 426 families, sisters and daughters who had ever used OCs were at significantly increased risk of breast cancer compared with sisters and daughters who had never used OCs (RR, 3.3; 95% CI, 1.6-6.7). The risk of breast cancer associated with OC use was not elevated among granddaughters, nieces, or marry-ins. The test for interaction between degree of relationship to the proband and OC use was statistically significant ($P=.03$). Although based on a relatively small number of cases, risk ratios did not significantly differ for any relationship category by duration of OC use (1-4 vs >4 years), by age at first use (≤25 vs >25 years old), by time since first use (≤10 vs >10 years), or by time since last use (≤10 vs >10 years; data not shown).

### Analyses in High-Risk Families

To study families most likely to be carrying a mutation in *BRCA1* or *BRCA2*, we conducted analyses in families defined as high risk by the number of breast and ovarian cancers among the blood relatives (Table 4). Among 132 high-risk families in which at least 3 blood relatives were diagnosed with breast or ovarian cancer, the interaction of OC use with degree of relationship reached even stronger statistical significance ($P=.006$) than in the entire cohort of 426 families. Among sisters and daughters in high-risk families, ever use was associated with an RR of 4.6 (95% CI, 2.0-10.7). Use of OCs

**Table 4.** Association of Oral Contraceptive (OC) Use With Risk of Breast Cancer, by Relationship to Proband, in High-Risk Breast–Ovarian Cancer Families*

| | | Entire Cohort (426 Families)† | | | ≥3 Breast or Ovarian Cancers (132 Families)‡ | | | ≥5 Breast or Ovarian Cancers (35 Families)§ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Relationship to Proband | OC Use | No. of Breast Cancers | Person-Years | RR (95% CI) | No. of Breast Cancers | Person-Years | RR (95% CI) | No. of Breast Cancers | Person-Years | RR (95% CI) |
| Sisters and daughters | Ever | 13 | 2533 | 3.3 (1.6-6.7) | 10 | 733 | 4.6 (2.0-10.7) | 6 | 326 | 11.4 (2.3-56.4) |
| | Never | 25 | 15063 | 1.0 | 16 | 5534 | 1.0 | 3 | 1991 | 1.0 |
| Nieces and granddaughters | Ever | 37 | 38178 | 1.2 (0.8-2.0) | 26 | 14885 | 1.2 (0.7-2.0) | 12 | 5048 | 1.4 (0.6-3.3) |
| | Never | 78 | 67522 | 1.0 | 61 | 29985 | 1.0 | 26 | 11210 | 1.0 |
| Marry-ins‖ | Ever | 26 | 33930 | 1.2 (0.8-1.9) | 26 | 33930 | 1.1 (0.7-1.8) | 26 | 33930 | 1.1 (0.7-1.8) |
| | Never | 60 | 67940 | 1.0 | 60 | 67940 | 1.0 | 60 | 67940 | 1.0 |

*RR indicates relative risk; CI, confidence interval.
†$P$ interaction <.05.
‡Families in which at least 3 blood relatives were diagnosed with breast or ovarian cancer. $P$ interaction ≤.01.
§Families in which at least 5 blood relatives were diagnosed with breast or ovarian cancer. $P$ interaction ≤.01.
‖Marry-ins are from all 426 families for all analyses.

by granddaughters, nieces, and marry-ins was not associated with significantly increased risk of breast cancer. When the analysis was limited to 35 very high-risk families in which at least 5 blood relatives were diagnosed with breast or ovarian cancer, the risk among sisters and daughters was even greater (RR, 11.4; 95% CI, 2.3-56.4).

Since defining high-risk families on the basis of the number of cancers does not take into account family size, we also calculated standardized incidence ratios. This was done by applying Iowa's 1973-1977 age-specific incidence rates for breast and ovarian cancer in white women to the age structure of the at-risk women. A family was defined as high risk for this analysis if at least 1 more case of breast or ovarian cancer was observed than was expected based on population incidence rates. This resulted in 98 families being classified as high risk. The RRs obtained in families defined as high risk according to this classification were in the same direction as when high risk was based on a simple count of the number of cancers in the family: 3.6 (95% CI, 1.5-8.7) for sisters and daughters, 1.0 (95% CI, 0.5-2.0) for granddaughters and nieces, and 1.1 (95% CI, 0.7-1.7) for marry-ins. When the analysis was conducted in 38 families with 2 excess breast or ovarian cancers, the RR of breast cancer among sisters and daughters who used OCs increased to 7.1 (95% CI, 2.5-19.7), and the RR among granddaughters and nieces increased to 1.7 (95% CI, 0.7-4.3). In these 38 families, adjustment for parity, age at first birth, age at menarche, age at menopause, oophorectomy, pack-years of smoking, and educational level decreased the RR for sisters and daughters to 5.2 (95% CI, 1.9-14.3) and increased the RR for granddaughters and nieces to 2.3 (95% CI, 0.8-6.2).

**Table 5.** Association of Oral Contraceptive (OC) Use Before and After 1975 With Breast Cancer Risk, by Closest Affected Relative*

| Closest Affected Relative and Period | OC Use | No. of Breast Cancers | Person-Years | RR (95% CI) |
|---|---|---|---|---|
| First-degree relative 1975 or earlier | No | 29 | 20 264 | 1.0 |
| | Yes | 16 | 3896 | 3.3 (1.5-7.2) |
| After 1975 | No | 43 | 23 231 | 1.0 |
| | Yes | 2 | 929 | 0.9 (0.2-4.5) |
| Second-degree relative 1975 or earlier | No | 75 | 67 213 | 1.0 |
| | Yes | 33 | 31 923 | 1.3 (0.8-2.0) |
| After 1975 | No | 103 | 86 661 | 1.0 |
| | Yes | 5 | 12 475 | 0.6 (0.2-1.3) |
| Marry-ins 1975 or earlier | No | 60 | 71 302 | 1.0 |
| | Yes | 26 | 30 568 | 1.2 (0.8-1.9) |
| After 1975 | No | 80 | 92 143 | 1.0 |
| | Yes | 6 | 9727 | 1.1 (0.4-2.6) |

*Women who used oral contraceptives both before and after 1975 contribute person-years to both groups. RR indicates relative risk; CI, confidence interval.

### Control for Potential Bias Due to Missing Data on OC Use

To determine if missing data on OC use might be biasing our results, we used a propensity score method to assign weights based on the probability of having nonmissing OC data. The Cox proportional hazards model of the interaction of OC use with relationship to the proband was refit for the entire cohort of 426 families using these estimated weights. People with a high probability of missing OC use were weighted more heavily in the Cox model because they were underrepresented in the cohort. Implementation of these weights had a minor influence on the results. The RR of breast cancer associated with ever use of OCs using this model compared with the unweighted model was 2.9 (95% CI, 1.3-6.5) compared with 3.3 among sisters and daughters, 1.3 (95% CI, 0.8-2.2) compared with 1.2 among granddaughters and nieces, and 1.1 (95% CI, 0.7-2.0) compared with 1.2 among marry-ins.

### Dates of OC Use

We investigated whether the elevated risk of breast cancer associated with OC use in sisters and daughters of the proband was the result of the greater likelihood that sisters and daughters were exposed to the earlier formulations of OCs that contained higher doses of estrogen and progestins. The amount of estrogen in OCs has decreased from an initial 150 µg to 50 µg or less currently, with concurrent decreases in the level of progestogens.[30] Although we collected data on the particular years of OC use, we did not ascertain exact formulations or dosages. With the data available, we examined the relationship between breast cancer risk and estimated years of exposure to high-dose and low-dose formulations. Since all OCs initially marketed after 1975 contain 50 µg or less of ethinyl estradiol and 1 mg or less of several progestins,[30] we used this year as the cut point. Results are presented by closest affected relative rather than by relationship to the proband to maximize statistical power (TABLE 5). Results were unchanged when analyses were conducted by relationship to the proband. No association was observed between OC use after 1975 and risk of breast cancer for any category of family history, although statistical power was limited (eg, only 2 cases among 60 exposed women with a first-degree family history of breast cancer). However, the risk of breast cancer associated with OC use before 1975 was elevated among women with a first-degree family history of breast cancer (RR, 3.3; 95% CI, 1.5-7.2), but not among women with a second-degree family history (RR, 1.3; 95% CI, 0.8-2.0) or among marry-ins (RR, 1.2; 95% CI, 0.8-1.9). Although statistical power was limited, the elevated risk among women with a first-degree family history did not appear to

be influenced by duration of pre-1975 OC use but did appear to persist for more than 10 years after last use of such formulations (data not shown).

If women with a family history are more likely to undergo screening mammography than are marry-ins, then surveillance bias could account for our findings. Indeed, the mean number of mammograms was higher among unaffected women with a first-degree family history than among unaffected women with a second-degree family history or unaffected marry-ins: 6.1, 4.3, and 4.4, respectively, after adjustment for age at interview. Moreover, the mean number of mammograms was slightly higher for OC users than nonusers overall (5.6 vs 4.3). After adjustment for total number of mammograms, the RR among women with a first-degree family history and pre-1975 OC use decreased to 2.4 but remained statistically significant.

## COMMENT

Our results suggest that the use of OCs in women with a strong family history of breast cancer may further elevate their breast cancer risk. Sisters and daughters of probands who had ever used OCs had a more than 3-fold increase in risk of breast cancer compared with similarly related women who had never used OCs. The risk was further elevated when analyses were conducted in high-risk families. The elevated risk of breast cancer was most pronounced for women with a first-degree family history of breast cancer who used OCs before 1975. However, the mean age at interview for those who used OCs after 1975 was only 43 years (range, 26-67 years).

We expected the risk of breast cancer associated with OC use among women with a second-degree family history of breast cancer to fall somewhere between that of first-degree relatives and marry-ins. Although this was not evident in the entire cohort of 426 families, there was some suggestion of an increased risk among second-degree relatives when the analyses were conducted in high-risk families and adjustment was made for other breast cancer risk factors. The lack of substantial evidence for an increased risk in the second-degree relatives may be due to the younger age of these women. The mean age of the granddaughters at the time of interview was only 45.3 years.

To our knowledge, this study is the first to examine the association of OC use with risk of breast cancer within the context of a multigenerational family study. Previously it was recommended that women with mutations in *BRCA1* or *BRCA2* consider OC use to reduce their risk of ovarian cancer.[19] Although our findings are not directly comparable since we did not analyze DNA for these mutations in all cases, the results seen in our highest risk families suggest that women with a strong genetic predisposition may be at greatly elevated risk of breast cancer if they use OCs. Effective prevention against ovarian cancer is certainly desirable given the high mortality associated with this malignancy and the difficulty of early detection. However, breast cancer is more common than ovarian cancer in these high-risk families. Additional evidence that women at high risk should avoid OC use comes from a recent study that suggests that OCs may increase the risk of breast cancer more in carriers of *BRCA1* or *BRCA2* mutations than in noncarriers, although these results should be viewed with caution given the small sample size.[21]

We are not aware of any studies that have examined the risk of breast cancer associated with OC use classified according to hormone dose in women with a family history of breast cancer. Considering the years of ascertainment in most published studies that examined OC use and breast cancer risk by a family history of breast cancer, women could have been exposed to either low- or high-dose formulations or both. It is possible that this heterogeneity of exposure led to some of the inconsistencies observed in previous studies. Several studies, including the Nurses' Health Study[14,18] and the Cancer and Steroid Hormone Study[11,15] did not observe significantly increased risks of breast cancer associated with OC use among women with a family history of breast cancer. Our findings may have differed because our cohort is enriched for a family history of breast cancer. Other studies that have shown an increased risk of breast cancer associated with OC use include studies focusing on early onset cases with a first-degree family history of breast cancer (eg, UK National Case-Control Study Group[5]) and studies of known *BRCA1* or *BRCA2* mutation carriers.[21]

In vitro experiments on breast cancer cell lines have shown that wild-type *BRCA1* inhibits the transcription activity of the estrogen receptor-α under certain conditions.[31] Mutations in *BRCA1* may remove this inhibitory effect, thereby increasing estrogen-dependent epithelial proliferation in the breast. This proposed interaction between *BRCA1* and the estrogen receptor may contribute to the increased risk associated with OC use observed in some of our families.

The Minnesota Breast Cancer Family Study is a unique, well-defined resource for genetic epidemiologic studies that offered us several advantages in our analysis of OC use and breast cancer risk. The selection of the original breast cancer probands was essentially population-based. Participation rates by the families in this study have been very high (>93%), with an average of only 1 or 2 individuals per family lost to follow-up. The length of follow-up was extensive, on average more than 35 years, and as long as 64 years. We expect that recall of aspects of OC use that we analyzed (ever vs never use, total duration of use, and ages of use) in this population was accurate. Agreement between recalled history and records of prescribing gynecologists for these aspects of OC use has been shown to be reasonably good and nondifferential with regard to case and control status.[32]

Several complicating factors must be considered when interpreting the results of this study. Trends in OC use in the United States have been pronounced. Prevalence of OC use has increased markedly over time, especially among younger women. Total

duration of use has also increased. In addition, substantial changes in the type and concentration of the estrogen and progestin components of OCs have occurred since their introduction in 1960, from 150 µg of mestranol to 50 µg or less of ethinyl estradiol, and 9.85 mg of norethynodrel to 1 mg or less of several progestins.[30] The rising incidence of breast cancer over the years of follow-up further complicates the analysis. Although we adjusted for quartiles of birth cohort, we were unable to completely control for all temporal trends. Our estimation of low-dose vs high-dose formulations of OCs was based on use before or after 1975 since all formulations of OCs initially marketed after 1975 contain 50 µg or less of ethinyl estradiol and 1 mg or less of several progestins.[30] Therefore, some misclassification of high-dose vs low-dose exposure likely occurred. Since most instances of misclassification would result in individuals with low-dose exposure being classified as having high-dose exposure, we consider this to be a conservative approach.

Surrogate data on OC use were not collected due to their potentially low reliability. Therefore, data on OCs are limited to women who were alive and able to complete the telephone interview between 1991 and 1996. If OCs are associated with improved survival after breast cancer, one would expect to see an increased risk of breast cancer associated with OC use in this cohort. While some evidence exists for breast cancers in OC users being at an earlier stage, it is unknown whether this stems from earlier detection of breast cancer in these women, from the biological effects of the OCs, or from a combination of factors.[1] To help assess whether survivor bias was a concern in our study, we compared the length of time from breast cancer to interview among OC users and nonusers. After adjustment for birth cohort, the mean survival time was not significantly different between OC users (12.0 years) and OC nonusers (11.9 years), $P = .92$. In addition, the RR of breast cancer associated with OC use among the marry-ins in our cohort is comparable with published estimates in general population samples.[1]

The possibility of surveillance bias, specifically whether OC users and women with a family history of breast cancer had more frequent mammograms and therefore were more likely to have a breast cancer detected, was addressed by adjusting the model of pre- and post-1975 use for total number of mammograms. In this model, the risk among women with a first-degree family history who used OCs before 1975 was attenuated (RR, 2.4 vs 3.3) but still significantly increased. Therefore, surveillance bias does not appear to strongly affect our observations.

An important advantage of this study population is the complete knowledge of the sampling frame. Even when family members had died, we had knowledge of their existence and obtained selected data on these women as well as on living women who were unable to complete a telephone interview. This information was used to try to control for potential bias due to missing data on OC use. Implementation of weights based on the probability of non-missing data on OC use had a negligible impact on the results; thus, the absence of data on OC use among selected women was an unlikely explanation for our findings.

In summary, women with a first-degree family history of breast cancer who used OCs prior to 1975 were at significantly increased risk of breast cancer. We saw no evidence for an increased risk of breast cancer associated with use of OCs after 1975 in first-degree relatives, second-degree relatives, or marry-ins. However, only 60 women with a first-degree family history of breast cancer used OCs after 1975 and only 2 of these were diagnosed with breast cancer, so our estimated RR is somewhat unstable for this group of younger women. Also, because of the potential for misclassification of exposure, we are hesitant to draw conclusions about the influence of more recent OC formulations on breast cancer risk in women with a first-degree family history of breast cancer. Further follow-up is needed to investigate any association between current formulations of OCs and breast cancer incidence in these high-risk women. In addition, we will be completing *BRCA1* and *BRCA2* mutation screening in the high-risk families to determine whether these or other genes are responsible for the modifying effect of family history on the association between OC use and breast cancer. Women who have a first-degree family history of breast cancer and OC exposure may want to be particularly vigilant regarding appropriate breast cancer screening practices.

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As no two persons are exactly alike in health so neither are any two in disease; and no diagnosis is complete or exact which does not include an estimate of the personal character, or the constitution of the patient.

—Sir James Paget (1814-1899)

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**Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women.** *R. Gershoni-Baruch[1,2], E. Dagan[1, 2], D. Israel[3], E. Friedman[3].* 1) Dept Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Bruce Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa, Israel; 3) Susanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Center, Tel-Aviv, Israel

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5 methyltetrahydrofolate the primary circulatory form of folate and carbon donor for the re-methylation of homocysteine to methionine. A common missense mutation (C677T) in the MTHFR gene is associated with reduced enzyme activity, hyperhomocysteinemia and increased risk for atherosclerosis. Recently, a marginal association of the C677T polymorphism with endometrial and colorectal cancer was observed. To delineate the putative role of the C677T polymorphism in breast/ovarian tumorogenesis we determined the frequency of this polymorphism in 491 Jewish women with either sporadic (n = 355), hereditary (n = 136) breast and/or ovarian cancer who were all previously genotyped for the three predominant Jewish founder mutations in BRCA: 185delAG, 5382insC and 6174delT. Sixty nine asymptomatic BRCA mutation carriers were similarly analyzed. We found that C677T homozygotes were equally distributed among women with either sporadic (71/355; 20%) or hereditary breast/ovarian cancer (43/ 205; 21%); among women diagnosed with breast cancer prior to age 42 (22/122; 18%) and after that age (42/243; 17.3%); and among BRCA mutation carriers either asymptomatic (11/69; 15.9%) or manifesting cancer (32/136; 23.5%). Among women with bilateral breast cancer and those with both breast and ovarian carcinomas the rate of C677T homozygotes (24/72; 33.3%) was significantly higher (p = 0.0026). This observation, namely, that C677T homozygotes are at greater risk of acquiring a second primary tumor, if further corroborated has important clinical implications.

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**A large genomic deletion of *hMLH1* in a family with Muir-Torre syndrome.** *J.J.P. Gille[1], M.H.P. Strunk[1], R.J. van Schooten[1], L. Jaspars[2], M.H. Vermeer[3], G. Pals[1], F.H. Menko[1].* 1) Dept. of Clinical Genetics and Human Genetics; 2) Dept. of Pathology; 3) Dept. of Dermatology, University Hospital Vrije Universiteit, Amsterdam, The Netherlands.

Muir-Torre syndrome (MTS) is an autosomal dominant condition characterized by sebaceous gland tumors and visceral malignancies. In kindreds diagnosed with HNPCC (hereditary nonpolyposis colorectal cancer) sebaceous gland tumors and other MTS-associated skin tumors have been recognized. HNPCC is often due to germline mutations in one of five DNA-mismatch repair (MMR) genes (*hMSH2, hMLH1, hPMS1, hPMS2,* and *hMSH6*). Among MTS kindreds 14 *hMSH2* and 2 *hMLH* have been reported in the literature. Evidently, MTS and HNPCC are overlapping syndromes.

We studied a family (C149) in which the index patient (II-1) had two primary colorectal cancers at the age of 32 years. His father (I-1) had recurrent skin lesions diagnosed as sebaceous adenomas, sebaceous epitheliomas, keratoacanthomas with sebaceous differentiation and squamous cell carcinoma. At the age of 58 years this latter patient developed colonic cancer. No other close relatives were diagnosed with large bowel cancer or skin tumors. MSI studies of the colonic tumors of both patients revealed the MSI-H (high) phenotype. Germline mutation analysis of *hMLH1* and *hMSH2* by single strand conformation analysis and direct sequencing revealed that I-1 was apparently homozygote for two frequently occurring *hMLH1* polymorphisms located in exon 8 (676AG) and intron 14 (IVS14-19AG), respectively. Surprisingly, II-1 was not a carrier of any of these two polymorphisms, indicating that both patients were in fact hemizygote and carriers of a (partial) deletion of the *hMLH1* gene. Hemizygosity was confirmed by analysis of CA-repeat markers intragenic (D3S1611) and closely linked to *hMLH1* (D3S2623). No transmittance of alleles from I-1 to II-1 was observed. Our results indicate that both affected relatives are carriers of a genomic deletion of *hMLH1* that encompasses at least exons 8-14. The family presented here is the first MTS family with a large genomic deletion of *hMLH1*.

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**Mutational analysis of the RET proto-oncogene in 200 French MEN 2 families: a genotype-phenotype correlation.** *S. GIRAUD[1], P. PIGNY[2], P. NICCOLI-SIRE[3], P. WIZARD[1], A. MURAT[4], M. BILLAUD[5], G.M. LENOIR[1], GETC[3].* 1) Lab. de Genetique,Hospital E. Herriot,LYON,FRANCE; 2) Hospital Huriez,LILLE; 3) Hospital La Timone,MARSEILLE; 4) Hotel-Dieu,NANTES; 5) CNRS,UMR 5641,LYON.

Germline mutations of the RET proto-oncogene are associated with three inherited related disorders: multiple endocrine neoplasia type 2A (MEN 2A), type 2B (MEN 2B) and familial medullary thyroid carcinoma (FMTC). We have screened exons 8, 10, 11, 13, 14, 15 and 16 of RET in the germline DNA of 200 MEN 2 families. RET mutations have been identified in 99% of MEN 2A (101/102), 100% of MEN 2B patients (27/27). Mutations of RET were found in 91% of FMTC families (66/72) but in all FMTC families with at least three cases of MTC.The majority of MEN2A mutations identified in our series were missense changes located in the region coding for the extracellular cysteine-rich domain of RET: 86% of the mutations affected codon 634 in exon 11 and 10% involved either codons 609, 611, 618 or 620 in exon 10. Also, two single nucleotide substitutions were found in exons 13 and 14 (Y791F and V804M) in two MEN 2A cases. A unique mutation in exon 16 (M918T) within the RET tyrosine kinase has been identified in all cases. With regard to FMTC, mutations in exons 10 et 11 were found in 54%; of the cases. However, as previously described, the distribution of mutations was dissimilar to MEN 2A since cysteine codons of exons 10 and 11 were affected in 39% and 15%, respectively. Furthermore, a new RET mutation that consists in a nine base pair duplication in exon 8 which creates an additional cysteine codon was characterized in one FMTC kindred. Finally, point mutations at codons that specify residues within the tyrosine kinase domain were found in 35% of the cases: 8% at codon 768 or 790 in exon 13; 20% at codon 804 in exon 14 and 7% at codon 891 in exon 15. Notably, carriers of RET mutations in exons 13 to 15 were characterized by a later age of onset and a variable penetrance of medullar thyroid cancer.Finally, based on the results of our functional analyses we will propose a possible biochemical explanation for the correlation between genotype and phenotype.

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**Renal Neoplasms in a Familial Multisystem Syndrome with Fibrofolliculomas as a Cutaneous Marker.** *G.M. Glenn[1], M.M. Walther[1], J.R. Toro[1], S. Hewitt[1], P. Duray[1], P.L. Choyke[2], G. Weirich[3], M. Turner[1], W.M. Linehan[1], B. Zbar[3].* 1) Genetic Epidemiology Branch, Urologic Oncology Branch, Dermatology Branch, and Laboratory of Pathology, National Cancer Institute, Bethesda, MD; 2) Diagnostic Radiology Department; National Institutes of Health, Bethesda, MD; 3) Laboratory of Immunobiology, Frederick Cancer Research and Development Center, Frederick, MD.

In our studies of familial kidney neoplasms, we recognized a subset of families with renal tumors who were also affected by lung cysts, pneumothorax, and multiple cutaneous papules. In some family members, skin examinations, biopsies and dermatopathologic diagnoses were consistent with Birt-Hogg-Dube syndrome (BHD), a dominantly inherited predisposition to developing fibrofolliculomas, trichodiscomas, and acrochordons, but previously not known to be associated with internal neoplasms. We found renal neoplasms and BHD segregated together in an autosomal dominant pattern. To identify internal tumors, we performed CT scans of abdomen and pelvis with contrast, high resolution chest CTs, renal sonograms, and now have added colonoscopies to improve ascertainment of cases in families for linkage analysis. With referrals from dermatologists nationwide and abroad, we are studying 23 families with 79 individuals affected with BHD, of which 20 have renal epithelial neoplasms, 19 have spontaneous pneumothorax histories, and 12 have had colon polyps and/or colon carcinoma, and a colon tubulovillus adenoma has been seen. Distribution of renal tumor (RT) number in individuals from BHD families is: 1 RT in each of 6 individuals; 2-3 RTs in 4 individuals; and greater than 2-3 RTs in 10 individuals. Renal histopathologies included: Renal oncocytoma in 10 patients; papillary renal carcinoma in 4 patients; clear cell renal carcinoma in 4 patients; and chromophobe renal carcinoma in 2 patients. It is important to recognize the risk for benign and malignant internal tumors, and pneumothorax in individuals when there is a dermatologic diagnosis of BHD. The number and size of families we are studying should allow demonstration of the phenotypic spectrum and identification of the genetic basis of this genodermatosis associated multisystem and neoplastic syndrome.



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**Association of oral contraceptives with breast cancer risk in a population-based sample of 426 breast cancer families.** *D.M. Grabrick, J.R. Cerhan, F.J. Couch, R.A. Vierkant, T.M. Therneau, C.M. Vachon, J.E. Olson, V.S. Pankratz, L.C. Hartmann, T.A. Sellers.* Mayo Clinic, Rochester, MN.

Oral contraceptives (OCs) are weakly associated with an increased risk of breast cancer (BC) in the general population, but some data suggest a higher risk among BRCA1 and BRCA2 mutation carriers. This is clinically important as women in breast-ovarian cancer families may consider OC use to reduce their ovarian cancer risk. We analyzed data from the Minnesota Breast Cancer Family Study, a historical cohort study of relatives of 426 BC cases identified between 1944 and 1952, and followed through 1996. Ninety-eight percent of eligible families were recruited, and 93% of members participated. OC use and cancer incidence in sisters, daughters, granddaughters, nieces, and marry-ins were determined through telephone interviews. Through 1996, a total of 239 incident BCs were identified in the cohort of 6,150 women at risk. The lifetime prevalence of ever having used OCs was 51% overall and was similar for blood relatives and marry-ins (p=0.99). We used proportional hazards regression, accounting for birth cohort and correlated family data, to model the association between a time-dependent definition of OC use and age at onset of BC. The association of OC use with BC was examined within strata defined by degree of relationship to the proband, with never users as the reference category within each stratum. Among sisters and daughters, women who used OCs for 1 to 4 years were at 4.2-fold greater risk (95% C.I.: 2.1-8.6); for duration of use greater than 4 years the risk estimate was 2.2 (95% C.I.: 0.8-6.4). The corresponding risk estimates for granddaughters and nieces were 1.3 and 1.2, and for marry-ins 1.1 and 1.3, all nonsignificant. When analyses were repeated among the subset of families with 3+ breast or ovarian cancers, risks associated with OC use were further elevated among first-degree relatives: 5.5 (2.4-12.5) and 3.3 (1.0-11.0) for 1 to 4 and greater than 4 years, respectively. These data suggest that use of oral contraceptives may significantly increase risk of breast cancer among women with a family history of breast cancer, especially those with a strong family history.

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**Constitutional chromosomal instability and predisposition to childhood solid tumors; a new syndrome?** *B. Hirsch[1], S. Berry[1], B. Bostrom[1,2], S. Sencer[2].* 1) Univ Minnesota Medical School, Minneapolis, MN; 2) Children's Hospitals and Clinics, Minneapolis,MN.

The association between chromosomal instability (CI) and predisposition to malignancy is well documented in a number of genetic disorders. However, there are currently only a few well defined syndromes in which CI data are integrated into diagnostic testing or therapy planning.

We here report four children, from three unrelated families, who may represent a novel genetic syndrome. Clinical findings include IUGR, microcephaly, skin pigmentation anomalies, and/or anal abnormalities. Three children (CIA1, CIB1,CIC2)developed Wilms tumors within the first 2 yrs.of life,and one child (CIC1, the older sibling of CIC2),a high grade astrocytoma at 2.5 yrs. After surgical resection, chemotherapy was given to all but CIC2. Two children succumbed to therapy-associated AML within one yr., one died from therapy associated pancytopenia and sepsis. CIC2, who was not given chemotherapy because of concern for hypersensitivity, is alive 5 months post surgery. The three who succumbed all received topoisomerase II inhibitors.

G-banded metaphase analysis from blood lymphocytes and/or skin fibroblasts revealed markedly elevated rates of chromosomal breaks and rearrangements, 50 fold or greater relative to laboratory norms. No recurring abnormality or breakpoint was detected between children; however "clonal" rearrangements were found within individual studies. The pattern and rates of CI were not characteristic of a known disorder. SCE rates were normal, i.e. not indicative of Bloom syndrome.

The etiology of this syndrome is unknown. No mutation of the NF1 gene in patient CIAC2 was found. Although there was no prior significant cancer history in these families, analyses of mismatch repair genes are planned. Constitutional CI is clearly a hallmark of this disorder. Recently, a sibling to CIB1 was born with IUGR,microcephaly and displaced anus. Cytogenetic analysis revealed marked CI, as a result of which this patient is being carefully monitored for tumor development.

# ASSOCIATION OF ENDOGENOUS CORRELATES OF HORMONAL EXPOSURE WITH BREAST CANCER RISK IN 426 BREAST CANCER FAMILIES

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Previously we reported a 3.3-fold increase in breast cancer risk associated with use of early formulations of oral contraceptives in women with a strong family history of breast cancer, but little excess risk in women without such a family history. To determine whether endogenous correlates of estrogen and/or progesterone exposure influence risk in a similar manner, we examined the effects of age at menarche, age at menopause, parity, and age at first birth on breast cancer risk. The study population included 426 families ascertained through probands diagnosed with breast cancer between 1944 and 1952 at the University of Minnesota Tumor Clinic. Data were collected between 1991 and 1996 through telephone interviews of 395 sisters and daughters, 3,014 nieces and granddaughters, and a control group of 2,768 women who married into the 426 families. The participation rate was 93%. Relative risks and 95% confidence intervals were calculated using Cox proportional hazards regression and accounted for birth cohort, the non-independence of observations within a family, and other important breast cancer risk factors. No statistically significant interactions were observed between relationship to proband and any of the reproductive factors examined. Compared to less than 12 years, an age at menarche of 12-14 years (RR=0.42; 95% C.I.: 0.21-0.84) or greater than 14 years (RR=0.27; 95% C.I.: 0.08-0.84) was associated with significantly decreased risk of breast cancer among sisters and daughters, but not among nieces and granddaughters or among marry-ins. Menopause after age 50 years (versus at or before age 50) was associated with significantly increased breast cancer risk among nieces and granddaughters (RR=2.37; 95% C.I.: 1.44-3.90), but not among sisters and daughters (RR=1.46; 95% C.I.: 0.72-2.93) or among marry-ins (RR=1.22; 95% C.I.: 0.72-2.08). When parity and age at first birth were examined concurrently (with nulliparous women as the reference group), there were no striking differences between relatives and marry-ins. Our findings suggest that these correlates of endogenous hormonal exposure influence risk of breast cancer similarly among women with and without a strong family history of the disease.